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Association of menopausal characteristics and risk of coronary heart disease: a pan-European case-cohort analysis

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Genetically determined reproductive aging and cardiovascular risk factors and coronary heart disease risk: a two-sample Mendelian Randomization study

Veerle Dam[1,2], N Charlotte Onland-Moret*[1], Stephen Burgess[3,4,5], Maria-Dolores Chirlaque[6,33], Sanne A E Peters[1,7], Ewoud Schuit[1], Kaja Tikk[8,9], Elisabete Weiderpass[10,11,12,13], Clare Oliver-Williams[4,5], Angela M Wood[4], Anne Tjønneland[14,15], Christina C Dahm[16], Kim Overvad[16,17], Francoise Clavel-Chapelon[18], Heiner Boeing[19], Antonia Trichopoulou[20,21], Pietro Ferrari[22], Giovanna Masala[23], Claudia Agnoli[24], Rosario Tumino[25], Giuseppe Matullo[26,27], Salvatore Panico[28], Jolanda M A Boer[29], W M Monique Verschuren[1,29], Marit Waaseth[30], Virginia Menéndez[31], Miguel Rodríguez-Barranco[32,33], Olatz Mokoroa[33,34], Conchi Moreno-Iribas[35], Olle Melander[36], Sophia Harlid[37], Maria Nordendahl[38], Timothy J Key[39], Elio Riboli[40], Carmen Santiuste[33,41], Tilman Kühn[42], Verena Katzke[42], Claudia Langenberg[43], Nicholas J Wareham[43], Heribert Schunkert[44,45], Jeanette Erdmann[46], Christina Willenborg[46], Christian Hengstenberg[47], Marcus E Kleber[48], Graciela Delgado[48], Winfried März[48,49,50], Stavroula Kanoni[51], George Dedoussis[52], Panos Deloukas[51,53,54], Majid Nikpay[55], Ruth McPherson[55], Markus Scholz[56,57], Andrej Teren[57,58], Adam S Butterworth[4], Yvonne T van der Schouw[1]

*Corresponding author

[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands

[2]Netherlands Heart Institute, Utrecht, the Netherlands

[3]MRC Biostatistics Unit, University of Cambridge, Cambridge, United Kingdom

[4] MRC/BHF Cardiovascular Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom

[5]Homerton College, Hills Rd, Cambridge, United Kingdom

[6]Department of Epidemiology, Regional Health Authority, IMIB-Arrixaca, Murcia University, Murcia, Spain

[7]The George Institute for Global Health, University of Oxford, Oxford, United Kingdom

[8]Division of Clinical Epidemiology and Aging Research, German Cancer Research Centre (DKFZ), Heidelberg, Germany

[9]German Cancer Consortium (DKTK), German Cancer Research Centre (DKFZ), Heidelberg, Germany

[10]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway

[11]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

[12]Genetic Epidemiology Group, Folkhälsan Research Center and Faculty of Medicine, University of Helsinki, Helsinki, Finland

[13]Department of Research, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway

[14]Danish Cancer Society Research Center, Copenhagen, Denmark

[15]Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[16]Department of Public Health, Aarhus University, Denmark

[17] Department of Cardiology, Aalborg University Hospital, Denmark

[18]INSERM, Centre for Research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones, and Women's Health Team, Institut Gustave Roussy, Villejuif, France

[19]Department of Epidemiology, German Institute of Human Nutrition (DIfE), Potsdam-Rehbrücke, Germany

[20]Hellenic Health Foundation, Athens, Greece

[21]WHO Collaborating Center for Nutrition and Health, Unit of Nutritional Epidemiology and Nutrition in Public Health, Dept. of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece.

[22]International Agency for Research on Cancer, Lyon, France

[23]Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network - ISPRO, Florence, Italy

[24]Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

[25]Cancer Registry and Histopathology Department, 'Civic - M.P. Arezzo' hospital, ASP Ragusa, Italy

[26]Department of Medical Sciences, University of Torino

[27]Italian Institute for Genomic Medicine –IIGM/HuGeF, Italy

[28]Dipartimento di medicina clinica e chirurgia, Federico II University, Naples, Italy

[29]National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

[30]Department of Pharmacy, Faculty of Health Sciences, UiT the Arctic University of Norway, Tromsø, Norway

[31]Public Health Directorate, Asturias, Spain

[32]Escuela Andaluza de Salud Pública. Instituto de Investigación Biosanitaria ibs.GRANADA, Universidad de Granada, Granada, Spain

[33]CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

[34]Public Health Division of Gipuzkoa, Biodonostia Research Institute, San Sebastian, Spain

[35]Instituto de Salud Pública de Navarra, IdiSNA, Navarre Institute for Health Research, REDISSEC Pamplona, Spain

[36]Department of Clinical Sciences, Malmö, Lund University, Malmö

[37]Department of Radiation Sciences, Oncology, Umea University, Umea, Sweden

[38]Department of Public Health and Clinical Medicine, Umea University, Umea, Sweden

[39]Nuffield Department of Population Health, University of Oxford, Oxford, England

[40]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom

[41]Department of Epidemiology, Murcia Regional Health Authority, IMIB-Arrixaca, Murcia, Spain

[42]Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Foundation under Public Law, Heidelberg, Germany

[43]MRC Epidemiology Unit, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom

[44]Deutsches Herzzentrum München, Technische Universität München, Munich, Germany

[45]DZHK (German Center for Cardiovascular Research), partner site Munich Heart Allicance, Munich, Germany

[46]Institute for Cardiogenetics, University of Lübeck, Lübeck, Germany

[47]Department of Internal Medicine II, Division of Cardiology, Medical University of Vienna, Vienna, Austria

[48]Vth Department of Medicine, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

[49]Synlab Academy, Synlab Holding Deutschland GmbH, Mannheim, Germany

[50]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

[51]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, United Kingdom

[52]Department of Nutrition-Dietetics/Harokopio University, Athens, Greece

[53]Centre for Genomic Health, Queen Mary University of London, London EC1M 6BQ, United Kingdom

[54]Princess Al-Jawhara Al-Brahim Centre of Excellence in Research of Hereditary Disorders (PACER-HD), King Abdulaziz University, Jeddah 21589, Saudi Arabia

[55]Ruddy Canadian Cardiovascular Genetics Centre, University of Ottawa Heart Institute, Ottawa, Canada

[56]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Germany

[57]LIFE Research Center for Civilization Diseases, University of Leipzig, Leipzig, Germany

[58]Heart Center Leipzig, Leipzig, Germany

Corresponding author:

Dr. N. Charlotte Onland-Moret

n.c.onland@umcutrecht.nl

Huispostnummer STR. 6.131

P.O. Box 85500

3508 GA Utrecht

the Netherlands

Abstract

Background: Accelerated reproductive aging, in women indicated by early natural menopause, is associated with an increased risk of coronary heart disease (CHD) in observational studies. Genomic variants for age at natural menopause (ANM) have been implicated in genome stability, immune function and mitochondrial biogenesis, which are not sex-specific processes. We aimed to establish the causal association between reproductive aging and (non-)fatal CHD and CHD risk factors using ANM variants as a measure for genetically determined reproductive aging in women and in men, since genome-wide association studies (GWAS) for reproductive aging traits in men are lacking.

Methods: We performed a 2-sample Mendelian Randomization (MR) using four methods: the simple median-based method, the weighted median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression. Summary statistics were pooled from three studies with together 417,579 participants from European descent, including 49,150 CHD cases. Publicly available GWAS and EPIC-CVD were pooled for total cholesterol, high density lipoprotein cholesterol, triglycerides, HbA1c, and glucose.

Results: Our MR analyses show no association between genetically determined reproductive aging and CHD risk in women (Relative Risk Estimate $(RRE)_{IVW}$=0.99, 95% confidence interval (CI): 0.97;1.01), or any of the CHD risk factors. No associations were found in men.

Conclusion: Reproductive aging is not causally associated with CHD risk or CHD risk factors in women, nor in men. The association between early menopause and CHD risk in observational studies might be the result of residual confounding, reverse causation, or reflect a shared aetiology that results in both earlier menopause and higher CHD risk.

Keywords: Reproductive aging, Mendelian randomization, coronary heart disease, cardiovascular risk factors

Key messages:

- Genetically determined reproductive aging is not associated with coronary heart disease in women.
- Genetically determined reproductive aging is not associated with coronary heart disease in men, although the validity of the genetic instrument is not established in men.
- Genetically determined reproductive aging is not associated with cardiovascular risk factors (total cholesterol, high density lipoprotein cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B, C-reactive protein, glucose and HbA1c).

Introduction

Cardiovascular disease (CVD) is the leading cause of death in both men and women(1). Accelerated reproductive aging, as indicated by early menopause in women, has been associated with increased risk of CVD(2–5). The mechanisms underlying these associations are not fully understood yet; deterioration of traditional CVD risk factors, in particular cholesterol, has been suggested to play a role(6,7). Although men do not experience an abrupt start or stop of their reproductive period, there is limited evidence that in men reproductive functions, such as erectile dysfunction, sperm motility and morphology, and semen volume, also decline with aging(8–10). Some of these, e.g. erectile dysfunction and decreasing testosterone levels, sometimes referred to as andropause, have been associated with increased CVD risk as well(11,12). Since male reproductive aging is a gradual process into old age, it is more complicated to study health effects of accelerated reproductive aging in males.

In observational studies, it is difficult to disentangle the potential independent effect of accelerated reproductive aging on CVD risk from the effect of general aging, as residual confounding can still be present. Furthermore, reversed causality can also play a role here, as women with an unfavourable CVD risk profile have been reported to experience accelerated reproductive aging(13). Mendelian Randomization (MR) designs, exploiting the principle of random independent segregation of alleles at meiosis, are a means to establish causality in situations where randomized clinical trials are impossible(14,15). In MR studies, single nucleotide polymorphisms (SNPs) associated with the exposure as found in genome-wide association studies (GWAS) are used as instrumental variables.

To date, GWAS have been conducted for the reproductive aging trait age at natural menopause (ANM) in women, while GWAS for male reproductive aging traits are not available.

The ANM GWAS reported 56 SNPs that are mainly implicated in genome stability (DNA repair), immune function and mitochondrial biogenesis(16). As these mechanisms are not specific for women, we hypothesized that these mechanisms underlie reproductive aging in men as well.

A recent study in three cohorts suggested a harmful effect of ANM, genetically determined by the 56 SNPs, on CVD and CHD risk in women, but not in men. However, the sample size was small. Replication in a large sample size using publicly available data, conducted in women only, gave a null finding (17). This study did not investigate cardiovascular risk factors as an outcome.

The aims of the present study are to establish the causal association between reproductive aging and fatal or non-fatal CHD, and to gain more insight in possible mechanisms underlying the association between genetically determined reproductive aging and cardiovascular risk factors in women, using 56 SNPs associated with earlier ANM. Furthermore, we aim to establish whether the same mechanisms are associated with CHD and traditional cardiovascular risk factors in men as well. We used the same 56 ANM variants as a measure for genetically determined reproductive aging in men, postulating common genetic mechanisms of reproductive aging.

Methods

Study populations and outcomes

Fatal or non-fatal CHD

We used data from 417,579 participants of European descent (including 49,150 CHD cases) from three studies: the UK Biobank(18), a modified version of the CARDIoGRAMplusC4D consortium (m-CARDIoGRAMplusC4D) since we could only include those studies that provided us with sex-specific summary data (Cardiogenics, Thiseas, AMC-PAS, Duke 2, CCGB 2, ITH 2, OHGS A2, OHGS B2, OHGS C2, Germifs I, Germifs II, Germifs III, Germifs IV, LIFE-Heart and LURIC(19)), and the EPIC-CVD case-cohort study(20). Details of the three studies (UK Biobank, m-CARDIoGRAMplusC4D and EPIC-CVD), including definitions of fatal or non-fatal CHD in each study, can be found in supplement 1.

Traditional CHD risk factors

For the associations between genetically determined reproductive aging and CHD risk factors, we again used data from EPIC-CVD and combined these with publicly available GWAS summary statistics of the Global Lipids Genetics Consortium(21) (total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides) and MAGIC(22,23) (HbA1c, fasting glucose). Details on these consortia can be found in supplement 1.We did not have access to sex-specific data for these risk factors. Therefore, we could only perform a pooled MR analyses for men and women combined.

Genotyping and SNP selection

Genotyping in the UK Biobank was performed using the Affymetrix UK BiLEVE Axiom array and the Affymetrix UK Biobank Axiom Array(18,24). The m-CARDIoGRAMplusC4D studies have used various genotyping methods as described previously(19). EPIC-CVD participants were genotyped with the Human Core Exome array, Illumina 660 Quad array, and Omni Exome Express array. The Global Lipids Genetics Consortium and MAGIC also used different assays as described previously(21–23).

A recent genome-wide meta-analysis identified 56 SNPs associated with younger ANM among European descendants, 54 common HapMap SNPs and two Exome chip SNPs(16). All SNPs passed the threshold of p<5e-6, but not all the threshold of p<5e-8. No linkage disequilibrium (LD) at R^2>0.9 was present among these 56 SNPs. Pleiotropic effects were investigated by searching the NHGRI-EBI GWAS Catalog(25) and Phenoscanner(26) for the SNPs or their proxies (R^2>0.8). We used the 56 ANM variants as a measure for genetically determined reproductive aging in both women, and in men, since GWAS for reproductive aging traits in men are lacking.

Statistical analyses

We verified whether the ANM variants were a valid instrument for the MR analysis in women by calculating the F-statistic according to the method described previously(27), using the SD (5.8 years) for ANM from the imputed data in the EPIC-CVD subcohort and the beta's for the ANM variants from the GWAS(16).

Regarding the outcome CHD, for UK Biobank and m-CARDIoGRAMplusC4D, odds ratios and standard errors for the SNP-CHD relations were derived through contact persons. For

EPIC-CVD, Prentice-weighted Cox proportional hazards regression adjusted for age, country, the first two principal components and array was used to calculate hazard ratios and standard errors for the EPIC-CVD case-cohort set. Regarding CHD risk factors, we derived effect estimates and standard errors for the cardiovascular risk factors (Global Lipids Genetics Consortium(21) for total cholesterol, HDL cholesterol and triglycerides, and MAGIC(22,23) for HbA1c and fasting glucose) using Phenoscanner(26). In the random subcohort of EPIC-CVD, we first imputed the missing observational data of EPIC-CVD (non-genetic data only) using multiple imputation with the MICE package in R(28) with 10 imputations and 50 iterations, including the CVD risk factors, SNPs and other baseline characteristics as predictors. Subsequently, we derived regression coefficients with linear regression in the subcohort only, separately in each imputation, using the same adjustments as for CHD. Thereafter we pooled the results with Rubin's Rule(29).

We performed a 2-sample MR using four separate methods to estimate causal effects for binary (CHD) and continuous (total cholesterol, HDL cholesterol, triglycerides, apolipoprotein A (apoA1), apolipoprotein B (apoB), C-reactive protein (CRP), glucose and HbA1c) outcomes: the simple median-based method, the weighted median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression using the 'Mendelian Randomization' package in R(30). The IVW provides a consistent estimate and assumes that all assumptions of the instrumental variable are met, the median based and MR-Egger methods provide estimates under weaker assumptions, with the MR-Egger additionally providing an intercept that represents the average pleiotropic effect(31,32). When unbalanced horizontal pleiotropy is absent, results of all methods are expected to be consistent(33). We first conducted sex-specific MR analyses for CHD in all three studies (UK Biobank, m-CARDIoGRAMplusC4D, EPIC-

CVD) separately. Subsequently, we pooled the estimates with a fixed effect model as is standard in MR studies. Similarly, MR analyses were performed for each cardiovascular risk factor in each study separately (EPIC-CVD, Global Lipids Genetics Consortium, MAGIC) and then pooled using a fixed effects model. Sex-specific analyses were possible in EPIC-CVD only, therefore we pooled the results for both sexes for combining with Global Lipids Genetics Consortium and MAGIC). All analyses were conducted with R version 3.2.0(34).

Results

Table 1 provides an overview of the numbers of cases and non-cases in UK Biobank, m-CARDIoGRAMplusC4D, and EPIC-CVD.

(Table 1 here)

The F-statistic for genetically determined reproductive aging in women was 93.7. Table 2 shows the results for the association between genetically determined reproductive aging and CHD per MR method stratified by sex and by study (UKBiobank, m-CARDIoGRAMplusC4D, and EPIC-CVD). In women, the IVW analyses in each study separately showed no causal association between genetically determined reproductive aging and CHD, nor when studies were pooled together (Relative Risk Estimate[RRE]$_{IVW}$=0.99; 95% confidence interval [CI]=0.97;1.01). The MR-Egger method indicated no pleiotropic effects (intercept=0.004, p=0.318) and resulted in an RRE of 0.97 (95%CI=0.94;1.02) in the pooled data. Similar results were found for men with a pooled RRE$_{IVW}$ of 1.00 (95%CI=0.97;1.02), also indicating no pleiotropic effects (RRE$_{MR-Egger}$=1.00 (95%CI=0.95;1.05), intercept=0.000, p=0.948).

(Table 2 here)

Table 3 shows the IVW results for the association between genetically determined reproductive aging and cardiovascular risk factors, with sex-specific estimates only from the EPIC-CVD subcohort and the sex-combined pooled estimates from both publicly available GWAS data and the EPIC-CVD subcohort. For each one-year decrease in genetically determined reproductive

aging, total cholesterol levels decreased with 0.025 mmol/L in women in IVW-analysis, however this was not statistically significant (95%CI= -0.056;0.005). Similarly, genetically determined reproductive aging was not causally associated with total cholesterol in men ($beta_{IVW}$=0.024 mmol/L, 95%CI= -0.011;0.059), nor in the pooled sex-combined results (pooled $beta_{IVW}$=-0.005 mmol/L, 95%CI= -0.007;0.017). Again, no pleiotropic effects were detected (supplement 2). Furthermore, no causal association was found for HDL cholesterol, triglycerides, ApoA1, ApoB, CRP, glucose, and HbA1c (table 3).

(Table 3 here)

Discussion

This study did not find a causal association between reproductive aging and CHD risk or CHD risk factors, including cholesterol levels, in women. Furthermore, this study does not provide evidence for a causal association between reproductive aging and CHD risk or CHD risk factors in men.

Strengths of this study are that, to the best of our knowledge, this is the largest MR study of associations between reproductive aging and CHD to date with 20,169 CHD events in women and 27,397 in men. We used several methods for MR analyses all yielding consistent results for the tested hypotheses, and in women the instrument we used was strong (F-statistic 93.7). Some limitations need to be acknowledged. First, we cannot establish whether the ANM risk score is a valid instrument for reproductive aging in men. The F-statistic is calculated using observed menopausal age in women, but men do not have a similar trait with an abrupt stop in reproductive potential. Since the SNPs we used are mainly implicated in mechanisms that are not specific for women, we hypothesized that there are common mechanisms of reproductive aging for women and men, and that, therefore, the same variants can be used as marker for genetically determined reproductive aging in men. However, it needs to be acknowledged that corresponding phenotypic traits in men need to be further investigated. Second, the GWAS on ANM included women with an ANM between 40 and 60 years only and therefore did not include women with an extremely early menopause (<40) or premature ovary insufficiency (POI). Most of the observational studies did include women with an extremely early menopause or POI, and two recent systematic reviews and meta-analyses of observational studies showed that POI is associated with both fatal and non-fatal CHD and CVD(35,36). Although we could not study an effect of extremely early menopause in our MR study, a recent GWAS on early menopause

revealed no new genetic variants for early menopause and showed that the genetic aetiology of early menopause overlaps with that of ANM. Thus early menopause is at least partly explained by the same polygenic variants as ANM(37). Third, our analyses with glucose were based on both fasting (MAGIC) and non-fasting estimates (EPIC-CVD). Although both are associated with an increased CVD risk(38,39) it might not be appropriate to combine them, since different SNPs might drive the association and underlying mechanisms could be different.

Our findings regarding CHD are partly in contrast with one previous study investigating the association between ANM SNPs and CHD death, which reported a significantly increased risk of CHD death with a weighted genetic risc score (wGRS) in women, but not in men(17). However, our findings are in line with those of the MR analysis in women, presented in the same paper, using CARDIoGRAMplusC4D data only, which was also null. The discrepancy between the wGRS and MR findings is potentially due to the fact that the wGRS analysis was adjusted for several known CVD risk factors (current smoking, body mass index, hypertension, type 2 diabetes, total cholesterol, and lipid treatment). This might induce a biased association between the genetic variant and the outcome through confounder(s), also known as collider bias(40,41). In addition, the number of cases used for the wGRS analyses was small (only 541 CHD deaths in women), so a chance finding cannot be ruled out either.

Our MR-study suggests that the association between genetically determined reproductive aging and CHD is not causal. However, most observational studies do find an association between early age at menopause and CHD in women. We suggest several explanations for this finding. First, observational studies are susceptible to residual confounding and reverse causation. It is possible that residual confounding is still present. Postmenopausal women are by definition older than premenopausal women, making it challenging to separate the effects of

biological aging from the various phases of the reproductive aging process. Hence, residual confounding due to age may still be present in observational studies. Second, reverse causation is another potential problem in observational studies. Although most studies assume that an early ANM increases CHD risk, it might be possible that an unfavourable cardiovascular risk profile, or accelerated vascular aging, causes an early ANM. One previous study showed indeed that higher cholesterol levels prior to menopause were associated with earlier menopause(13). However, another study found no association between premenopausal CVD and subsequent age at menopause(42). If anything, women who developed CVD before menopause had a lower risk of becoming postmenopausal than women without premenopausal CVD (HR=0.98 for CVD and HR=0.90 for MI), indicating that menopause occurred later in these women(42), but none of these results were statistically significant due to the small number of premenopausal cases.

MR uses SNPs, that are randomly assigned by birth, as instrumental variables, and as such provides a method to assess causality(43). However, an MR study makes several assumptions, that have to be taken into account(44). The first assumption is that the genetic marker is associated with the exposure. The SNPs used in our study were all associated with ANM at a p-value <5e-6 in the latest and largest GWAS(16) . As discussed above, this may not be true in men. The second and third assumptions are that the association between the genetic marker and the outcome is explained exclusively through the exposure of interest and is unconfounded. This is often referred to as the absence of pleiotropy, which means that the genetic variant is not associated with other phenotypes. Although our Phenoscanner search showed that a few of the SNPs are associated with age at menarche or sex hormone levels, and thus that some pleiotropy may be present, our MR-Egger analysis showed no indication of

pleiotropy, since all intercepts were zero or very close to zero and non-significant(32). We therefore assume that our results are not biased by pleiotropy.

In summary, we found no evidence that reproductive aging is causally associated with CHD and CHD risk factors in women, nor in men. The association between early menopause and CHD risk in observational studies might be the result of residual confounding, reversed causation, or reflect a shared aetiology that results in both earlier menopause and higher CHD risk.

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Conflict of interest statement

Dr. Clare Oliver-Williams received £1000 in prize money from Novartis. None of the other authors report any potential conflict of interest.

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